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GABAERGIC NERVE TERMINALS DECREASE IN THE SUBSTANTIA NIGRA FOLLOWING HEMITRANSECTIONS OF THE STRIATONIGRAL AND PALLIDONIGRAL PATHWAYS

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SUMMARY

Glutamic acid decarboxylase (GAD), the enzyme that synthesizes the neurotransmitter, GABA, was immunocytochemically localized in axon terminals as well as in small and medium-sized neurons of the rat substantia nigra. The pattern formed by GAD-containing axon terminals with the dendrites and somata of neurons in the substantia nigra was altered following ipsilateral hemitransections of the striatonigral and pallidonigral pathways. A marked reduction of GAD-positive terminals occurred throughout this brain region, but the ventral fifth of the pars reticulata showed a nearly normal pattern of GAD-positive axon terminals.

The results of this investigation are consistent with results from biochemical studies which have indicated that the striatonigral and/or pallidonigral pathways are GABAergic. In addition, these results suggest that the residual GABAergic terminals remaining after hemitransection are derived from intrinsic neurons of the substantia nigra.

INTRODUCTION

The existence of a striatonigral pathway arising from the neostriatum and terminating in the pars compacta and pars reticulata of the substantia nigra has been known for sometime[10,11,18,31]. More recently, a descending pathway to the substantia nigra has also been shown to arise from neurons in the pallidum[3,9,12,13,16,20]. Thus, both the striatonigral and pallidonigral pathways provide synaptic input to the

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neurons in the substantia nigra. Results from biochemical studies of this brain region indicate that GABA and its synthesizing enzyme glutamic acid decarboxylase (GAD) are contained predominantly within axon terminals of the neurons that give rise to the striatonigral and pallidonigral pathways[2,6,7,14,17,19,23,24,32]. In addition, results of physiological and pharmacological studies provide evidence that these pathways monosynaptically inhibit neurons within the substantia nigra and that this postsynaptic inhibition is mediated by GABA[5,26,34].

The results from a previous immunocytochemical study[29] have shown a high concentration of GAD-positive (GABAergic) nerve terminals in both the pars compacta and pars reticulata of the rat substantia nigra. Biochemical studies have demonstrated highly significant decreases in nigral GAD activity following hemitransections of the striatonigral and pallidonigral pathways, but it is not known whether GAD-containing axon terminals are decreased homogeneously or whether they are lost only from specific regions of the substantia nigra. An immunocytochemical localization of GAD can be used to determine such a topography of the GABAergic terminal loss that results from lesions of the descending input to the substantia nigra. In this report, we present immunocytochemical results indicating that the striatonigral and/or pallidonigral pathways give rise to a dense, GABAergic projection to the substantia nigra. In addition, our results show that some GABAergic terminals remain following hemitransection and suggest that some of these terminals arise from local circuit, GABAergic neurons.

METHODS AND MATERIALS

In this study, three albino rats were anesthetized and placed into a David Kopf stereotaxic instrument. Following exposure of the cranium, a dental drill was used to cut a 3 mm slit in the skull perpendicular to, and 2 mm away from, the midsagittal suture at A–P + 4.5 mm. A Bard-Parker No. 11 scalpel blade was inserted through this opening until it touched the base of the cranium. The scalpel was then moved back and forth so that the descending striatonigral and pallidonigral pathways would be interrupted without significant direct damage to the substantia nigra. The contralateral substantia nigra as well as unoperated rats from a previous study[29] were used as controls. Within 2–3 days following surgery, the operated rats displayed rotational movements toward the side of the lesion indicating that the descending pathways to the substantia nigra were interrupted[22,30,33]. These rats each had a 6-day postoperative survival time.

In another experiment, a lateral ventricle in each of two rats was injected with 10 μl of a colchicine solution (10 μg/μl saline, Sigma Chemical Co.) The colchicine was injected at a rate of 1 μl every 3 min over a 30 min period from a 10 μl syringe that was mounted on a micromanipulator. Colchicine was used to interrupt axonal transport and thereby produce detectable GAD concentrations within the somata and dendrites of neurons that normally show GAD localized to only their axon terminals[28]. The survival time for these rats was 48 h post-injection.

All operated rats were perfused through the left cardiac ventricle with a solution

containing 4% paraformaldehyde and 0.002% $CaCl_2$ in 0.12 M Millonig's phosphate buffer. The perfused rats were stored overnight at 4 °C, and the brains were removed from the cranium the following day. Following a 1.0 h rinse in buffer, the brains were immersed overnight in 30% sucrose solution, rapidly frozen with dry ice and sectioned at 30 μm in a cryostat. Nissl-staining of some sections from these specimens was done in order to determine the extent of the lesion. Other sections that contained the substantia nigra from both experimental and control specimens were placed into phosphate buffer for 24 h prior to incubation in the immunocytochemical reagents. Sections for immunocytochemistry were consecutively incubated for 1.0 h in the following solutions: normal rat serum, rabbit anti-GAD serum or non-immune (control) rabbit serum, goat anti-rabbit serum, peroxidase:antiperoxidase Fab complex, and 3,3'-diaminobenzidine tetrahydrochloride (Sigma) and 0.006% H_2O_2. Sections were rinsed in buffer for 2.5 h following each incubation. After the immunocytochemistry, sections were processed for light microscopic observation using previously described procedures[1,27,29].

RESULTS

Sections incubated in anti-GAD serum displayed dense concentrations of GAD-positive puncta homogeneously distributed throughout the entire substantia nigra of unoperated control specimens. Results of a previous study[29] have shown these structures to be axon terminals containing GAD-positive reaction product. These terminals were highly concentrated around dendrites in light microscopic preparations, and this was reflected in ultrastructural observations in that approximately 90% of all axon terminals found in contact with nigral dendrites were GAD-positive[29]. Furthermore, GAD-positive terminals formed axosomatic synapses with cell bodies throughout the substantia nigra. Sections of the substantia nigra that were incubated in non-immune (control) serum showed no specific staining[29].

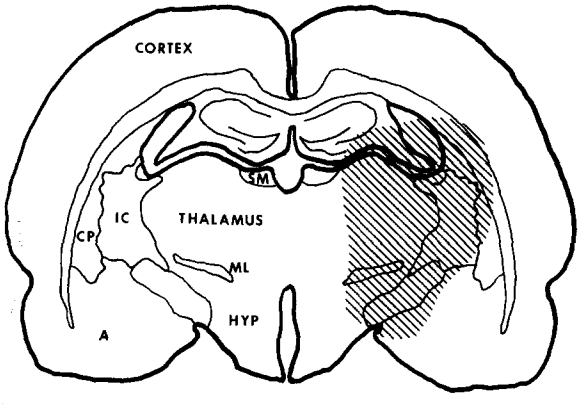
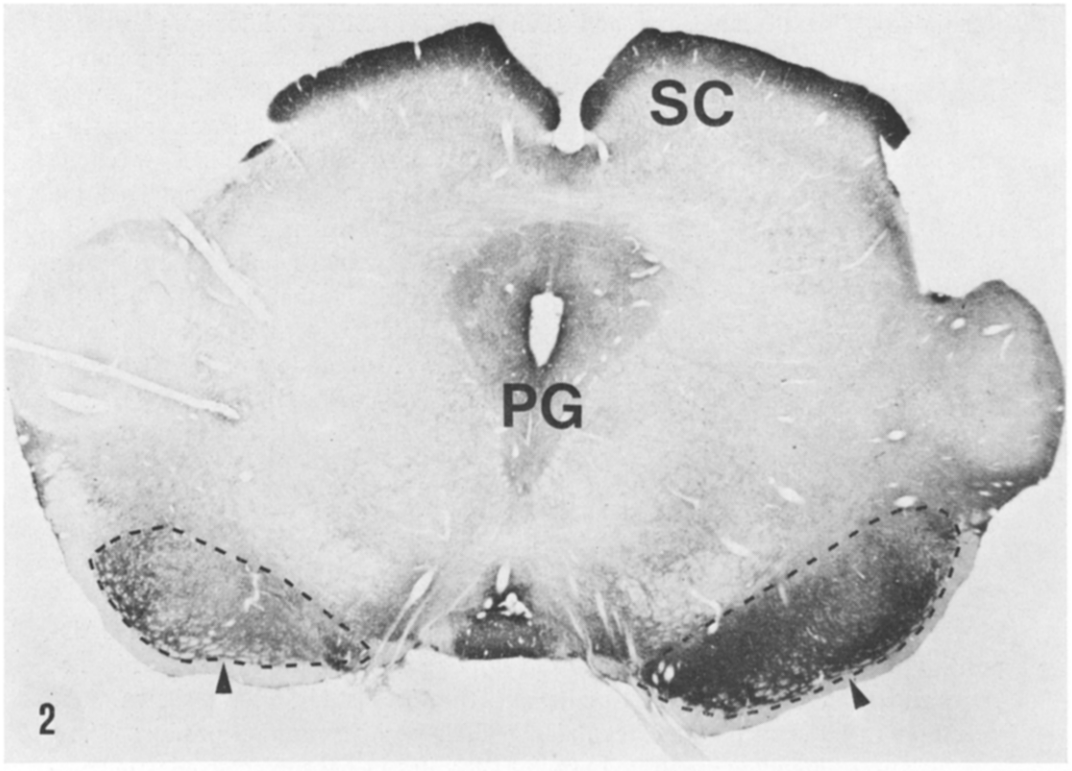


Fig. 1. Drawing of rat brain at the level of the hemitransection of the striatonigal and pallidonigral pathways. The lesion (diagonal lines) resulted in destruction of the internal capsule (IC) at the caudal part of the neostriatum (CP). Other structures damaged by the lesion include parts of the thalamus, hypothalamus (HYP), amygdala (A), medial lemniscus (ML), stria medullaris (SM) and the cerebral cortex (CORTEX). × 10.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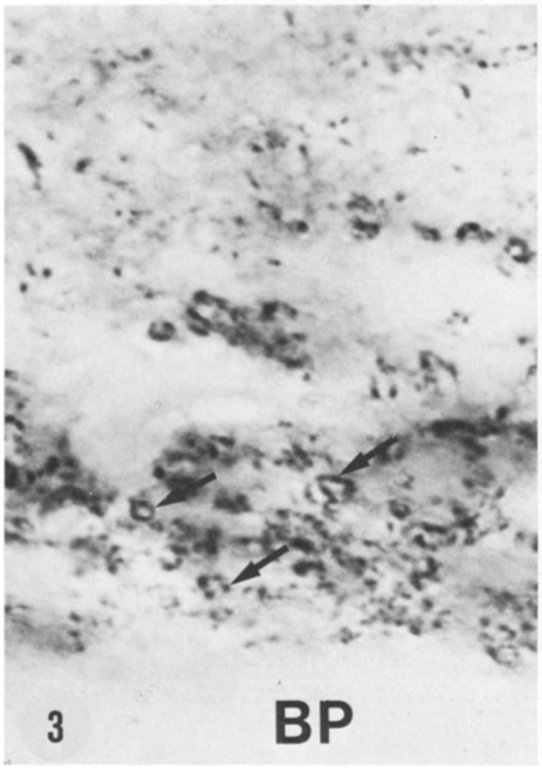
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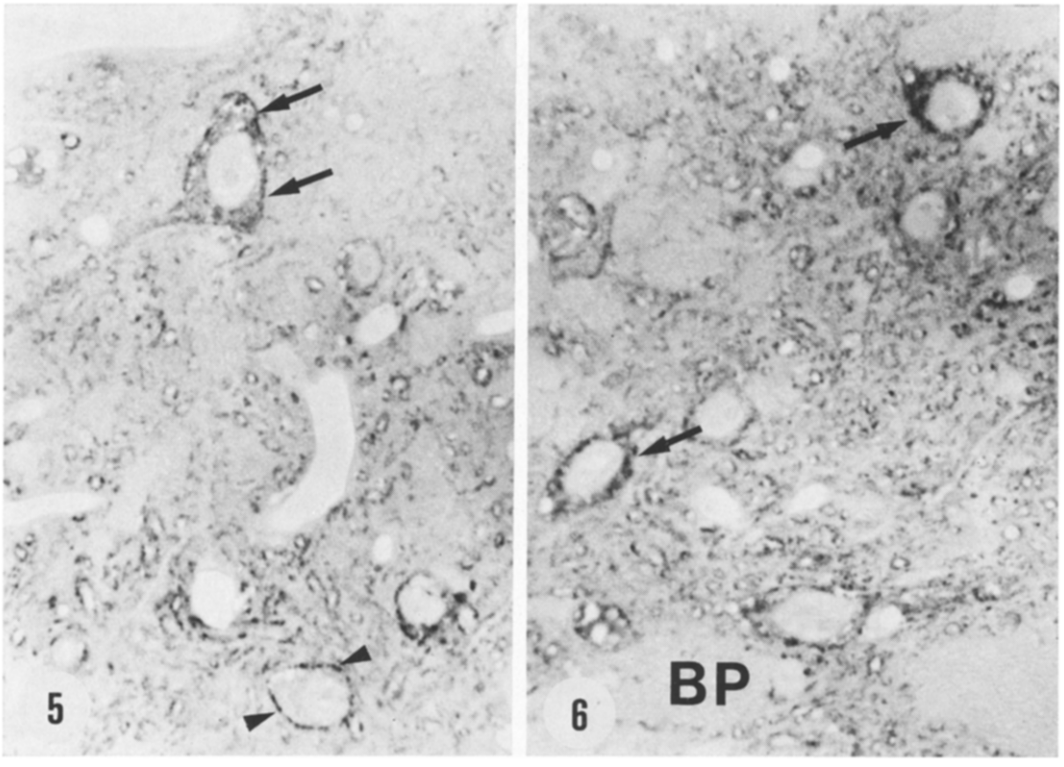

Figs. 5 and 6. Semithin, 1 μm sections from colchicine-injected rats.
Fig. 5. shows a medium-sized GAD-positive soma in pars compacta (arrows). Other somata are shown with GAD-positive axon terminals outlining their surfaces (arrowheads). These latter somata lack GAD-positive reaction product in their perikaryal cytoplasm. × 500.
Fig. 6. shows small GAD-positive somata (arrows) in pars reticulata. × 500.

In the hemitransected experimental specimens, histological observations of Nissl preparations confirmed the interruption of both the striatonigral and pallidonigral pathways at levels rostral to the substantia nigra (Fig. 1). Sections of the substantia nigra from all three experimental rats showed a large reduction of GAD-positive

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Figs. 2–4. Photomicrographs of frozen sections of the substantia nigra incubated in anti-GAD-serum reaction product.
Fig. 2. Brain stem section at the level of the superior colliculus (SC) shows dense deposition of GAD-positive reaction product in the substantia nigra (outlined) on the side contralateral to the hemitransection (right side) and reduced staining on the side ipsilateral to the hemitransection (left side). Note that no staining differences are present in either the superior colliculus (SC) or the periaqueductal gray (PG). × 15.
Fig. 3. At higher magnification on the side ipsilateral to the hemitransection (left arrowhead in Fig. 2), GAD-positive axon terminals (arrows) outline transversely-sectioned dendrites in the most ventral part of the substantia nigra, just dorsal to the basis pedunculus (BP). In more dorsal portions of the pars reticulata, the number of GAD-positive terminals is decreased greatly, and dendrites are not encircled by GAD-positive terminals. × 1000.
Fig. 4. On the side contralateral to the hemitransection (right arrowhead in Fig. 2), GAD-positive axon terminals (arrows) outline dendritic profiles throughout the pars reticulata. × 1000.

puncta on the side ipsilateral to the lesion (Fig. 2). This decrease of GAD-positive terminals occurred mainly in the central core of the substantia nigra, specifically in pars compacta and the dorsal 80% of the pars reticulata. The most ventral part of the pars reticulata and the lateral and medial poles of the substantia nigra were much less severely affected than the central core (Figs. 2 and 3). At higher magnification, GAD-positive terminals outlined dendritic profiles in the less severely affected regions, while in the central core of the substantia nigra this pattern was observed less frequently, and there were considerably fewer GAD-positive terminals (Fig. 3). The differences between normal and altered staining patterns are compared for the ventral pars reticulata in Figs. 3 and 4. On the side contralateral to the hemitransection (Figs. 2 and 4), the GAD staining in the substantia nigra was similar to that observed in sections from unoperated rats[29]. Thus, an interruption of the striatonigral and pallidonigral fibers caused a significant and topographically defined reduction of GAD-positive terminals in the substantia nigra.

In colchicine-injected rats, GAD-positive somata were observed throughout the substantia nigra. These neurons were identified in semithin, 1 μm plastic sections, and they corresponded to small and medium-sized neurons (Figs. 5 and 6) that were located in both pars compacta and reticulata.

DISCUSSION

The results of the hemitransection experiments indicate the presence of the neurotransmitter GABA in the axon terminals of the descending striatonigral and/or pallidonigral pathways. This finding is consistent with results from biochemical studies which have indicated that these pathways are GABAergic[2,5,6,7,13,14,17,19,23,24,25,32]. In addition, our results are consistent with morphological results showing that striatonigral terminals are preferentially distributed to pars reticulata[4] and pallidonigral terminals are mainly distributed to pars compacta[12] because a loss of GABAergic terminals was observed in both of these regions after hemitransections.

It is relevant to note that hemitransections did not cause a total loss of GAD-positive terminals in the substantia nigra. The terminals that remained in pars lateralis, may be due to a failure to transect fibers that arise from the caudal neostriatum and course via a supraoptic pathway caudal to the hemitransection[4]. Similarly, the terminals that remained in the medial part of the substantia nigra could be explained by a failure to interrupt fibers in the medial forebrain bundle that arise in the nucleus accumbens and adjacent neostriatum[4]. In contrast to these two loci of relatively unaltered numbers of GAD-positive terminals, those GABAergic terminals remaining in the most ventral pars reticulata are more difficult to explain. Fallon and Moore[4] have shown that the dorsal neostriatum projects to the ventral substantia nigra and the pathway taken by these fibers would most probably have been interrupted by the hemitransections. Therefore, another brain region of local circuit neurons could be responsible for the region of concentrated GABAergic axon terminals remaining in the most ventral pars reticulata of hemitransected specimens.

The most severe alteration of the substantia nigra following hemitransection

occurred in the central core where only a diffuse, homogeneous distribution of GAD-positive axon terminals remained. This pattern of staining could be due to axon terminals arising from GABAergic local circuit neurons because (1) results from the colchicine experiments indicate that some small and medium-sized neurons in the substantia nigra are GABAergic and (2) results from Golgi studies[15] show that small nigral neurons have local circuit projections throughout the substantia nigra and that medium-sized nigral neurons in pars reticulata give rise to local axon collaterals. Based on these data, it is suggested that the residual GABAergic terminals in the central core and the GABAergic terminals concentrated in the ventral pars reticulata are both derived from GABAergic local circuit neurons and/or collaterals of projection neurons. These interpretations are consistent with biochemical results that suggest the nigral GAD remaining after hemitransections is derived from intrinsic neurons[21,25]. Furthermore, they are in accord with the results of a recent pharmacological study by Grace and Bunney[8] showing that GABAergic cells in pars reticulata inhibit cells in pars compacta.

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